

Figure S4. Development of a novel primary keratinocyte cell engineering workflow Legends continue on next page

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a. Top: viable cell population as detected by SSC-A versus FSC-A flow cytometry 24 hrs post electroporation. Bottom: representative brightfield images.

b. Relative cell viability values based on Alamar Blue assay upon electroporation of OKC (crRNA targeting the *CCR5* locus).

c. Percentage of indels created 96 hrs post electroporation, measured through TIDE. Upper and lower whiskers represent the largest and smallest observed values within 1.5 times the interquartile range from the ends of the box.

d. Percentage of indels 4 days or 20 days post electroporation, measured through TIDE.

e. Percentage of indels created 96 hrs post electroporation at 1st or 2nd round of sequential editing, measured through TIDE. Upper and lower whiskers represent the largest and smallest observed values within 1.5 times the interquartile range from the ends of the box.

f. Flow cytometry data on uptake of ATTO-550 labeled Cas9:crRNA:tracrRNA by 6 x 106 primary foreskin keratinocytes 24 hrs post electroporation.

g. Flow cytometry data on uptake of ATTO-550 labeled Cas9:crRNA:tracrRNA by foreskin keratinocytes, tympanic membrane keratinocytes, and primary dermal fibroblasts 24 hours post-transfection.

h. Immunoblot analysis of 11q13 genes in TC-OKC 6 days post transduction with lentivirus.

i. *MIR548K* expression levels in OKC relative to control 96 hrs post transduction with lentivirus.